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NOTES ON GEOGRAPHIC DISTRIBUTION

Lepidocollema brisbanense (C. Knight) P.M. Jørg., a new record of a corticolous lichen (Pannariaceae, Peltigerales) for Indonesia

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Abstract. *Lepidocollema brisbanense* (C. Knight) P.M. Jørg. is reported as new to Indonesia. This species is characterized by foliose grey thallus with black rhizohyphae; apothecia with thalline margins, pale red-brown discs; ascospores simple, with a thin, smooth wall. Other details of the morphology, image of fresh material and microscopic characters are provided.

Key words. Cyanolichen, diversity, Halmahera Island, Malesia, North Moluccas

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INTRODUCTION

Lepidocollema Vain., a macrolichen genus, was first described by Vainio in 1890, within the Pannariaceae Tuck (Fungi, Ascomycota). It is the second-largest genus of Peltigerales group (Ekman et al. 2014). This genus is characterized by its corticolous, foliose thallus with a *Nostoc* as a photobiont, a gray thallus with flat rosettes, and a blackish hypothallus (Poengsungnoen et al. 2021). A recent molecular analysis found that *Lepidocollema* does not constitute a monophyletic group (Elvebakk 2021). However, there is a close affinity between this genus and *Physma* A. Massal (Ekman et al. 2014; Elvebakk et al. 2016). *Physma* is distinguished by the presence of thick--walled ascospores, whereas *Lepidocollema* is characterized by the presence of thin-walled ascospores (Ekman et al. 2014; Elvebakk 2021).

Lepidocollema is widely distributed, and according to the *Index Fungorum* (2024) and GBIF (2024), there are 24 accepted species distributed worldwide. Their distribution is mostly in the tropical region, but some also occur in subtropical regions, including Thailand (Rangsiruji et al. 2016; Poengsungnoen et al. 2021), Japan, India, La Réunion, Australia, the USA, Philippines, Brazil, Sri Lanka, Papua New Guinea, Guadeloupe (Ekman et al. 2014).

The diverse geographical and environmental conditions of the Indonesian islands explain their large, but still insufficiently known lichen biodiversity. The current focus of research on lichens is primarily centered on its diversity and potential as an environmental bioindicator, with extensive studies conducted in Sumatra and Java regions (Windadri 2019).

The North Moluccas, along with Maluku Province, are part of the Moluccas Islands and are within the central biogeographical region of Wallacea (van Welzen et al. 2005). Recognized as one of the world's 25 biodiversity hotspots, Wallacea supports highly diverse biological communities with numerous unique species (Myers et al. 2000; van Welzen et al. 2005; CEPF 2014), including a notable diversity of fungi. According to Retnowati et al. (2019), the lichen diversity recorded on these islands represents approximately 2.5% of the total species known from Indonesia. In the Moluccas, 35 lichen species from 11 families are commonly found, covering rocks, trees, and other surfaces in a range of colors and forms (Windadri 2019). However, data on lichen diversity in eastern Indonesia remains limited, which highlights the need for further investigation and exploration to develop a more comprehensive understanding of this region.

During our analysis of the materials obtained from the biological survey of the Moluccas Islands in 2022, a noteworthy lichen specimen was observed. To ascertain the identity of the intriguing lichen, we made a comprehensive comparison. Investigation of both morphological characters and molecular data has led to the discovery that the collection represents a new species, *Lepidocollema brisbanense* (C. Knight) P.M. Jørg., for Indonesia and specifically in the Moluccas Islands, which we document here. During this process, we also discovered another unpublished collection from Indonesia.

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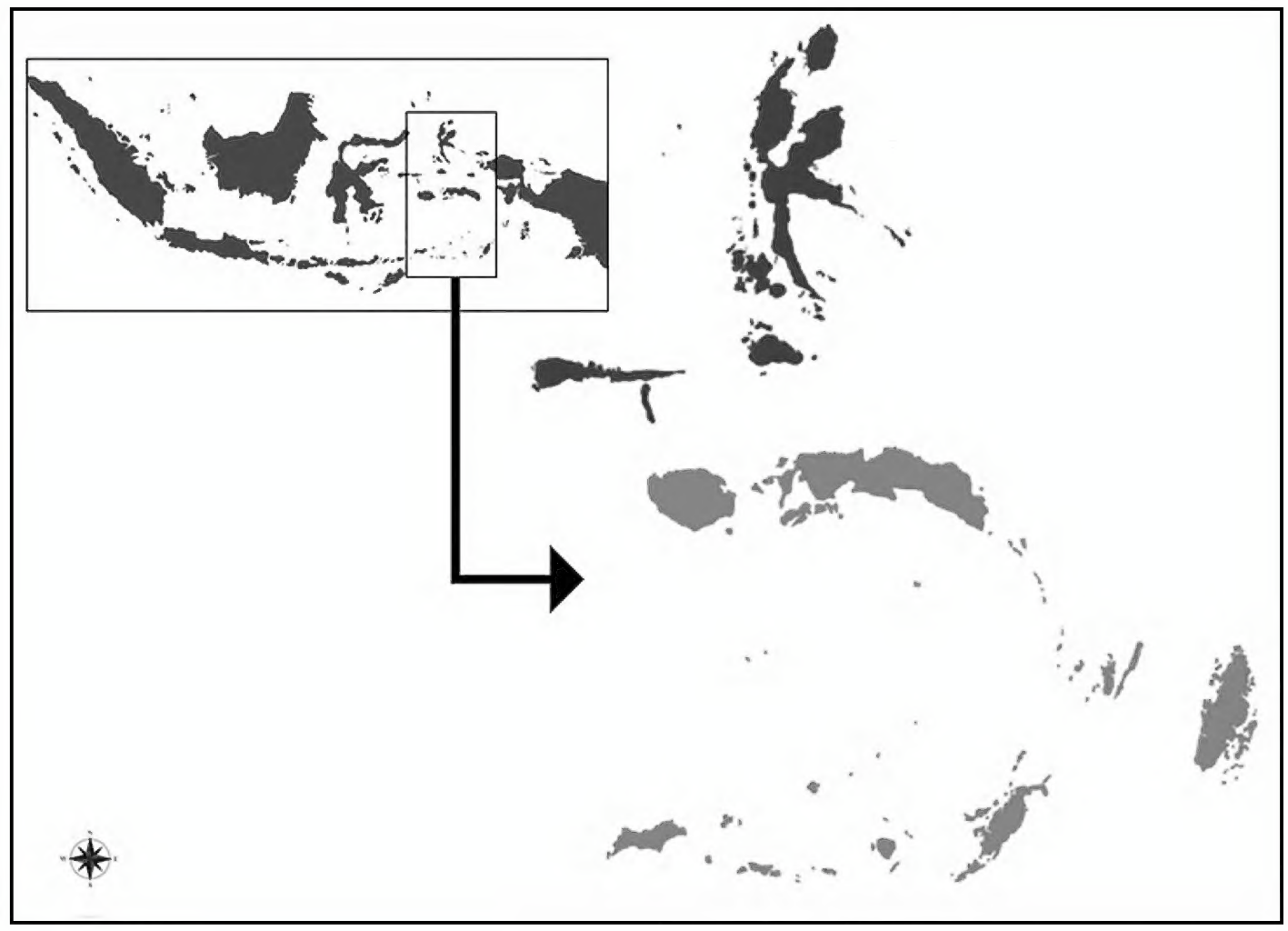

Figure 1. Maps of Moluccas showing the locality of *Lepidocollema brisbanense* in Halmahera Island.

METHODS

Material collection and observation. The specimen of *Lepidocollema brisbanense* was collected in September 2022 from the conservation area of Aketajawe-Lolobata National Park in Halmahera Island, Indonesia (Figure 1). Specimen was identified morphologically (thallus, ascocarps), anatomically (ascus, ascospores, hymenium), chemically (spot test: 10% KOH), and molecularly. Morphological characters were examined under a stereomicroscope, and anatomical characters were viewed under a compound light microscope. Additional herbaria data from GBIF and Meise Botanic Garden Herbarium (BR) were used to construct the species distribution in Indonesia.

DNA extraction, amplification and sequencing. For molecular analyses, fresh material from Halmahera Island was extracted and identified. Lichen DNA was extracted using the Plant Genomic DNA Mini Kit by Geneaid following the protocol manufacturer. The DNA amplification was conducted in a total volume of 30 µl consisting of 30 µg DNA, 1.125 µM each for ITS 1 F (Gardes and Bruns 1993) and ITS 4 for reverse (White et al. 1990), and 27 l KOD FX NeoToyobo/200 u (CAT. KFX-201). The PCR profile used for amplification was pre-denaturation at 95 °C for 5 min; denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, and 35× extension at 72 °C for 1 min; final extension at 72 °C for 5 min. The PCR product was submitted to 1st Base (Malaysia) via the service provided by PT. Genetika Science Indonesia for sequencing.

Phylogenetic tree construction. The obtained forward and reverse sequences data was processed to generate contig, which was subsequently subjected to analysis using the Basic Local Alignment Search Tool (BLAST) for species identification utilising the National Centre for Biotechnology Information (NCBI) website (González-Pech et al. 2019). Thirty sequences from the BLAST result (Table 1) were downloaded and aligned using the ClustalW method performed in MEGA v. 11 (Hung and Weng 2016; Tamura et al. 2021). The phylogenetic tree was constructed using the maximum-likelihood method (Keklik 2023). The maximum composite likelihood parameter model was utilised as suggested based on the dataset, and 1,000 bootstrap replications were performed to assess the robustness of the tree.

RESULTS

Phylogenetic analyses. According to the results obtained from the BLAST analysis of the GenBank nucleotide database, the sequences with the highest similarity were those of *Lepidocollema*. The specimen collected from Halmahera Island exhibited a genetic similarity of 98.92% with the reference sequence of *L. brisbanense* (KM887867.1) available in GenBank. Our specimen was classified as *L. brisbanense*, which is supported by a bootstrap value of 87, indicating a relatively high-level of confidence in the grouping (Figure 2).

Table 1. Specimens with GenBank accession numbers used for phylogenetic analysis, and the generated sequence of new record in bold.

Taxa	GenBank accession no.	Voucher	Origin
Collema flaccidum (Ach.) Ach.	MW462215	20072102	China
Collema furfuraceum (Arnold) Du Rietz	OQ917177	SNC171	USA, California
Collema furfuraceum Du Rietz	GQ396263	MA-16260	Spain, Madrid
Collema furfuraceum Du Rietz	MG552717	NY2044	Pakistan
Collema nigrescens (Huds.) DC.	OQ917178	SNC172	USA, California
Collema undulatum Laurer ex Flotow	MW462217	2019350	China
Collema undulatum Laurer ex Flotow	DQ466044	MA-Lichen 16036	Spain, Málaga
Lepidocollema brisbanense (C. Knight) P.M. Jørgensen	KM887867	NK-253	New Caledonia
Lepidocollema brisbanense	KT947068	RU0015RAMK	Thailand
Lepidocollema brisbanense	KT947066	RU0013RAMK	Thailand
Lepidocollema wainioi (Zahlbr.) P.M.Jørg.	KT947072	RU0048RAMK	Thailand
Lepidocollema brisbanense	PP711275	FSF40	Indonesia
Megalospora tuberculosa (Fée) Sipman	MH279966	KB377	Malaysia: Borneo
Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J.	KF704277	LG:T3	Thailand
Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J. Galloway	KF704278	LG:R1019	France, Reunion Island
Pannaria byssoidea Passo & Calvelo	MH802349	NK-100	Chile
Pannaria calophylla (Müll.Arg.) Passo & Calvelo	MH802350	NK-81	Chile
Pannaria conoplea (Ach.) Bory	AF429281	Ekman 3188 (BG)	Norway
Pannaria contorta (Müll.Arg.) Passo & Calvelo	MH802352	HSG080124-05	Chile
Pannaria durietzii (P.James & Henssen) Elvebakk & D.J.Galloway	MH802351	NK-04	New Zealand
Pannaria farinose Elvebakk & Fritt-Rasm.	MH802348	NK-19	Chile
Pannaria hookeri (Borrer) Nyl.	KY350562	NK-325	Norway
Pannaria hookeri (Borrer) Nyl.	AF429282	1989, Joergensen (BG)	Norway
Pannaria microphyllizans (Nyl.) P.M.Jørg.	AF429279	Kantvilas & Lumbsch 5601l (hb. Lumbsch)	Australia
Pannaria patagonica (Malme) Elvebakk & D.J.Galloway	MH802353	NK-416	Chile
Pannaria rubiginosa (Thunb. ex Ach.) Delise	AF429280	Anonby 870 (BG)	Norway
Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J. Galloway	KF704262	LG:R1247	France, Reunion Island
Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J. Galloway	KF704246	LG:T1	Thailand
Parmeliella brisbanensis (C. Knight) P.M. Jørg. & D.J. Galloway	KF704276	LG:T7	Thailand
Parmeliella mariana (Fr.) P.M.Jørg. & D.J.Galloway	KF704275	LG:R974	France, Reunion Island
Parmeliella stylophora (Vain.) P.M. Jørg.	KF704274	LG:R979	France, Reunion Island

***Lepidocollema brisbanense* (C. Knight) P.M. Jørg.**, Ekman, Wedin, Lindblom & Jørgensen, Lichenologist 46(5): 650 (2014)

Figure 3

≡ *Pannaria brisbanensis* C. Knight, Proc. Royal Soc. Queensland 6: 194 (1890); *Parmeliella brisbanensis* (C. Knight) P.M. Jørg. & D.J. Galloway, Flora of Australia, Vol. 54. Lichens – Introduction, Lecanorales 1 (Canberra): 314 (1992)

New record. INDONESIA – North Moluccas • Halmahera Island, Aketajawe-Lolobata National Park; 00°26′ 39.923″N, 127°44′25.871″E; 17.IX.2022; Fastanti 40 leg.; GenBank PP711275; BO 10642.

Characteristics. Thallus corticolous, squamulose to foliose, grey, with smooth surface, margin lobes elongate, 0.7–1 mm, with brown to black rhizohyphae below thallus protruding as a distinct peripheral prothallus zone. Photobiont cyanobacteria, cells globose 4–6 × 4–5 µm. Apothecia tight in middle of thallus, margin with thalline, flake, pale-red to brown disc when dried, width 0.5–2.0 mm. Hymenium hyaline, 60–80 µm long. Ascus contains 8 ascospores with thick apices, hyaline, 56.2–56.8 × 18.9–21.9 µm. Ascospores simple, ellipsoid, non-septate, 14.2–20.5 × 8.3–10.8 µm, with thin rings and slightly acute apical, brown to reddish, 0.9–1.6 × 2.1–3 µm in width.

Chemistry. K^-.

Distribution. Indonesia: Halmahera, Java, West Papua (Figure 4); Philippines (van den Boom et al. 2011; Paguirigan et al. 2020); Thailand (Rangsiruji et al. 2016); Vanuatu (Sipman 2018).

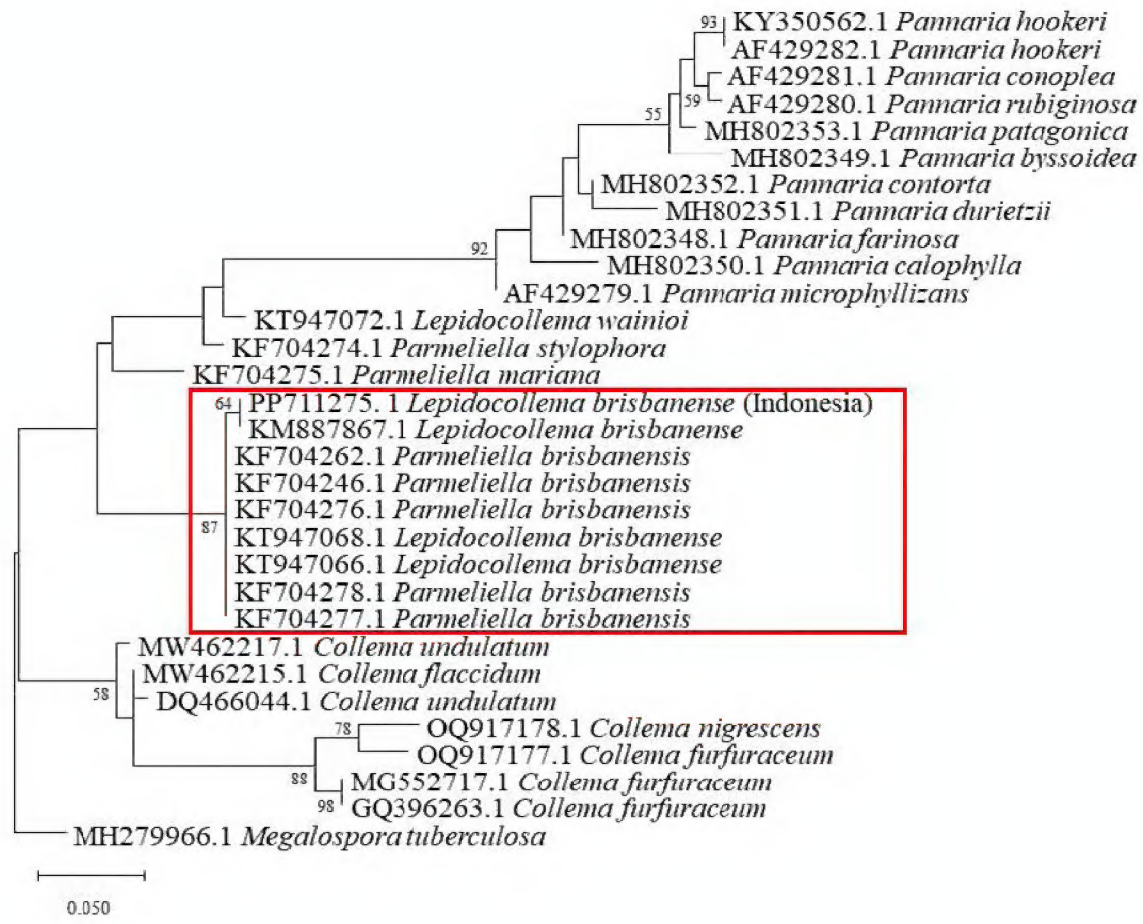

Figure 2. The phylogenetic tree and taxonomical position of *Lepidocollema brisbanense* from Indonesia (red box) were generated through the maximum-likelihood method, the maximum composite likelihood parameter model and 1,000 bootstrap replications.

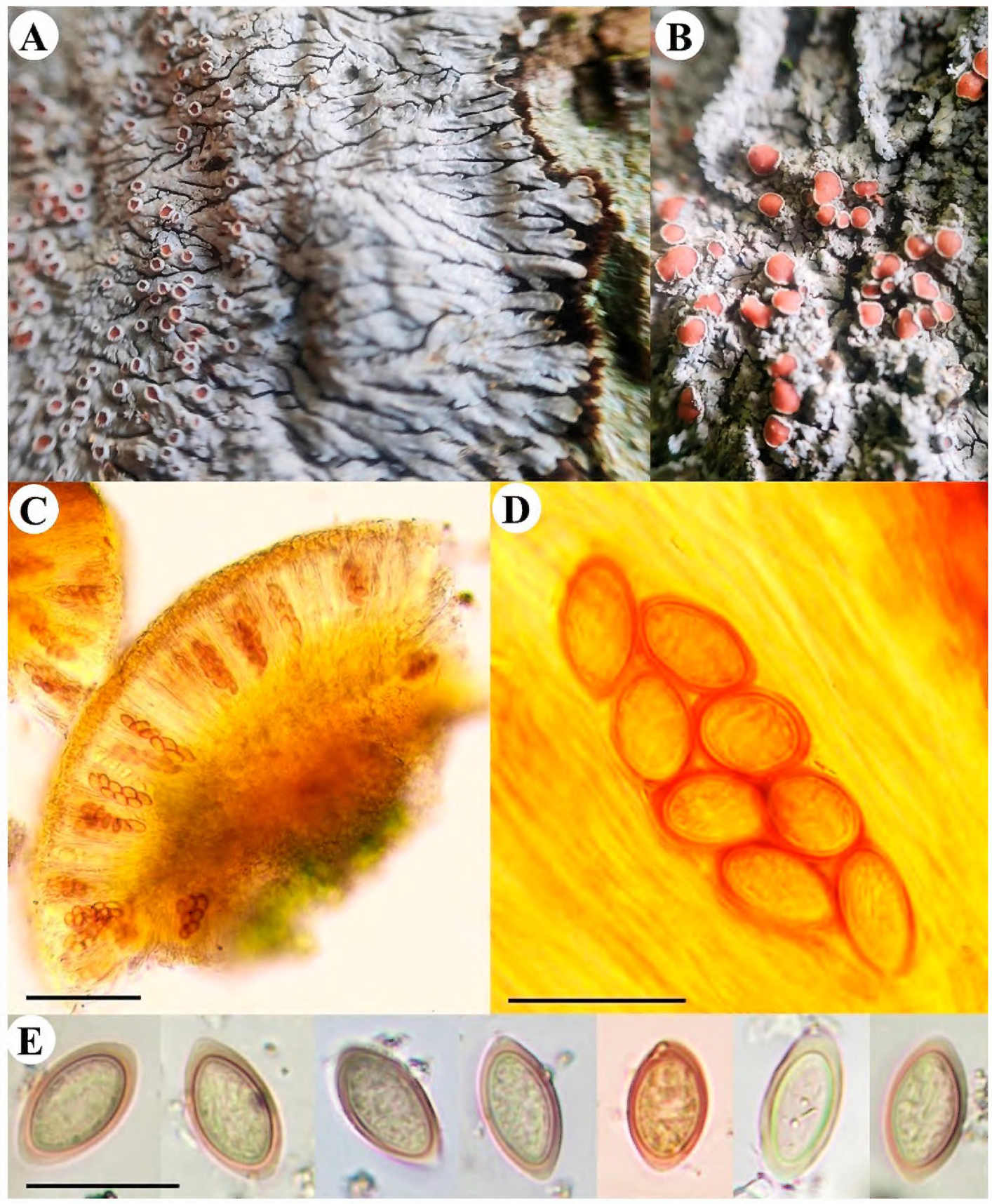

Figure 3. *Lepidocollema brisbanense*. **A.** Thallus with black rhizohyphae. **B.** Apothecia. **C.** Vertical section of apothecia. **D.** Ascus with 8 ascospores. **E.** Ascospores with perispore. Scale bars: C= 100 µm; D= 20 µm; E= 20 µm.

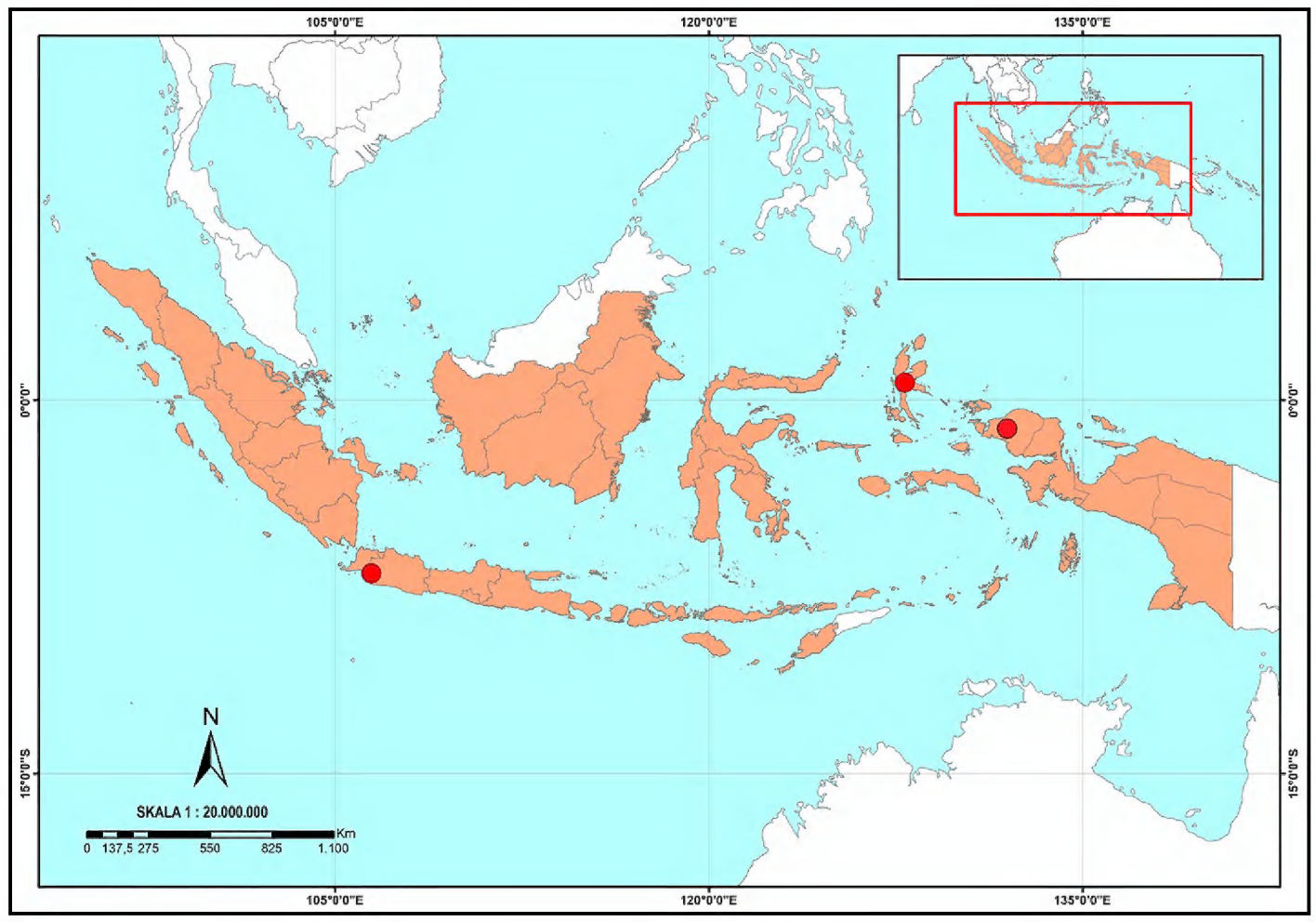

Figure 4. Distribution of *Lepidocollema brisbanense* in Indonesia: Java, North Moluccas, and West Papua (red dots).

Ecology. Corticolous, on a cocos palm tree (*Cocos nucifera* L.) near a river in a semishaded area at 50–60 m altitude.

DISCUSSION

Lepidocollema brisbanense is a corticolous lichen identified from Aketajawe-Lolobata National Park on Halmahera Island. Furthermore, we came across an unpublished record of this species based on a specimen in the Meise Botanic Garden Herbarium, as determined by Aptroot (Damien Ertz, pers. comm. 2023) and documented in the GBIF database. The data indicate that *L. brisbanense* is a species with no previous records reported in Indonesia. Based on our findings, the current range of *L. brisbanense* in Indonesia includes Java, North Maluku, and West Papua. This distribution is likely to be recognized in the future, given its prevalence in neighboring countries, such as Australia (Ekman et al. 2014), Thailand (Buaruang et al. 2017, Rangsiruji et al. 2016), and Philippines (van den Boom et al. 2011; Paguirigan et al. 2020).

Lepidocollema brisbanense exhibits distinct characteristics that facilitate its identification in the field. The characters are the presence of black rhizohyphae, a foliose thallus firmly attached to the substrate, a grey thallus with a lobus located at the apex of the thallus, and pale-red to brown apothecia. Additionally, the thallus is accompanied by simple ellipsoid ascospores with thin walls. This species is believed to have a close evolutionary relationship to *Lepidocollema marianum* (Fr.) P.M. Jørg., as evidenced by the presence of isidia or soredia on the thallus. According to Rangsiruji et al. (2016), *L. brisbanense* has isidia on the upper thallus surface, a trait is absent in *L. marianum*.

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ADDITIONAL INFORMATION

Conflict of interest

There are no competing interests.

Ethical statement

No ethical statement is reported.

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Author contributions
Conceptualization: FSF. Data curation: FSF, MRH. Formal analysis: FSF, MRH. Funding acquisition: FSF, AN, AR. Methodology: FSF, AN, MRH. Resources: FSF, AN, S. Supervision: FSF, AR, MRH. Visualization: FSF, MRH. Project administration: FSF, AN. Writing – original draft: FSF, MRH, AN, AR. Writing – review and editing: FSF, AN, MRH, AR, S.

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Data availability
All data that support the findings of this study are available in the main text.

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